

# Resistance to lysis of erythrocytes containing haemoglobin C—detected in a differential white cell counting system

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**SUMMARY** Erythrocytes containing haemoglobin C do not lyse normally in the peroxidase channel of the Technicon H6000 automated cell counter. This interferes with the normal function of the channel and results in a characteristic abnormal pattern. This correlates with a reduced osmotic fragility of the red cells.

A Technicon H6000 automated cell counter has been in routine use in the haematology laboratory of the Royal Berkshire Hospital, Reading since March 1982. The machine performs an automated differential white cell count by a mechanism which relies on prior lysis of erythrocytes. If this fails to be achieved completely then an erroneous result is derived. Although an infrequent occurrence, we have found that a very high proportion of patient samples demonstrating this phenomenon contain haemoglobin C.

## Material and methods

Blood samples anticoagulated with EDTA were analysed using a Technicon H6000 automated cell counter (Technicon Instruments Company Ltd). In addition to deriving red cell and platelet indices this machine incorporates two white cell counting channels. The erythrocytes are lysed in both prior to cytochemical staining of the white cells and subsequent analysis according to scatter and absorbance of light in optical systems. One channel employs an alcian blue stain to distinguish the basophil population; the other links a dye to a leucocyte peroxidase mediated reaction thereby distinguishing the remaining major white cell groups. Different methods of red cell lysis are used in the two channels; a mixture of polyoxyethylene lauryl ether (Brij 35) and sodium dodecylsulphate causes lysis in the peroxidase channel while cetylpyridinium chloride has this role in the basophil channel.

Haemoglobin electrophoresis was performed on relevant samples using cellulose acetate strips and

Tris EDTA glycine buffer, pH 9.2.

Osmotic fragility of the red cells was also assessed where possible, using the method of Parpart.<sup>1</sup>

## Results

A normal "scattergram" of white cell distribution as displayed in the Technicon H6000 peroxidase channel is shown in Fig. 1. In circumstances of increased erythrocyte resistance to lysis there may be incomplete red cell lysis in this channel. As a result the machine records a falsely high total white cell count and an abnormal distribution pattern in the peroxidase channel. Neither is reflected in the basophil channel where lysis has always been observed to be complete. This is easily verified from the appropriate function monitor trace on the machine. Of the first 28 000 patient samples analysed by the Technicon H6000 cell counter in our laboratory 22 showed incomplete red cell lysis in the peroxidase channel. The Table indicates the nature of these samples. It can be seen that a high proportion (17 of 22 cases) contained haemoglobin C.

The failure of red cell lysis is more marked in patients with homozygous haemoglobin C and

*Incidence of incomplete red cell lysis (of approximately 28 000 patient samples tested)*

	<i>No of samples</i>
Haemoglobin C trait	12
Haemoglobin SC	3
Haemoglobin CC	2
Haemoglobin SS*	2
Haemoglobin AS/ $\beta$ -thalassaemia trait	1
Cord blood samples	2

\*Failure of red cell lysis is unusual with HbSS; most cases lyse normally.

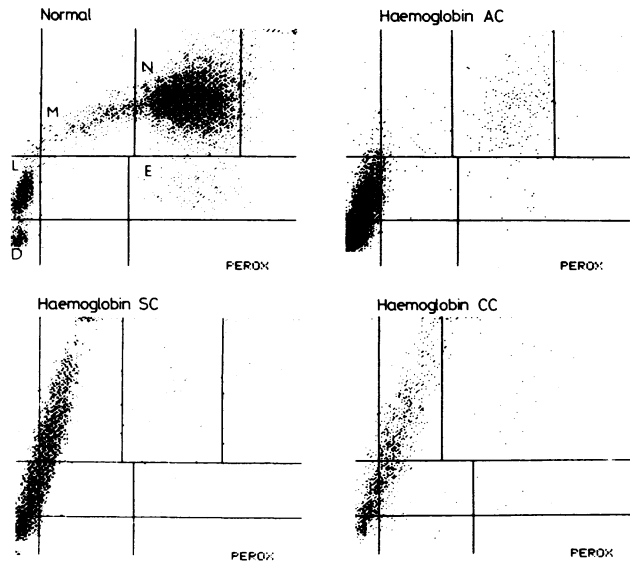


Fig. 1 Technicon H6000 peroxidase channel graphic display appearances (scattergrams). Distribution of leucocytes is illustrated in the normal display: N = neutrophils; M = monocytes; L = lymphocytes; E = eosinophils; D = red cell debris and platelets. Typical appearances of three samples containing haemoglobin C are shown. Failure of erythrocyte lysis results in a large cloud of small cells, poorly stained by the peroxidase-linked dye and interpreted as "lymphocytes". In more extreme instances (haemoglobins SC and CC) the cloud extends upwards, probably as a result of the greater "coincidence" of the more numerous unlysed cells. If failure of lysis is sufficiently marked (as here with haemoglobin CC) the relative paucity of leucocytes compared to unlysed red cells results in a failure of the machine to identify the correct neutrophil cloud.

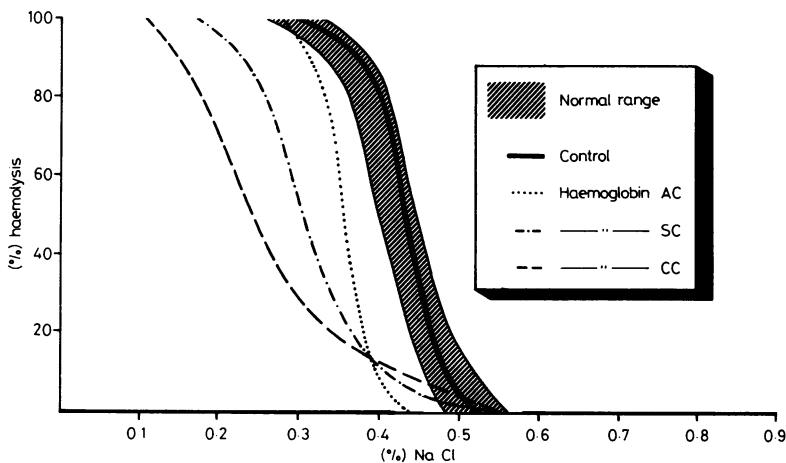


Fig. 2 Erythrocyte osmotic fragility curves demonstrating increased resistance to lysis in cases of haemoglobin C trait (AC), haemoglobin SC disease (SC) and homozygous haemoglobin C (CC).

haemoglobin SC disease compared to those with haemoglobin C trait. This results in different scattergram appearances (Fig. 1) allowing these two groups to be distinguished. The other conditions cited in the Table have shown patterns similar to haemoglobin C trait.

We have assessed osmotic fragility of the red cells in seven of the patients who have shown the phenomenon of incomplete erythrocyte lysis. In every case so far tested there has been a marked shift in the erythrocyte fragility curve towards increased resistance to lysis (Fig. 2).

In our laboratory haemoglobin electrophoresis is carried out routinely on blood samples received from any patient for whom sickle haemoglobin screening is requested, or who is known to be from an ethnic background where there is a significant likelihood of variant haemoglobins being detected. In addition, samples are electrophoresed if the blood film reveals any suspicion of a haemoglobinopathy. Despite this extensive screening 8/17 cases of haemoglobin C discovered during the period of this survey were found incidentally as a result of non-lysis of red cells in the Technicon H6000. Every specimen tested in the H6000 which has been known or subsequently shown to contain haemoglobin C (trait, disease or interaction with other haemoglobin) has shown resistance to red cell lysis in the peroxidase channel sufficient to cause a characteristic abnormal pattern.

## Discussion

The early studies on characterisation of haemoglobin C included demonstration of the fact that erythrocytes containing this variant haemoglobin showed increased resistance to osmotic lysis.<sup>2</sup> Gottfried and Robertson,<sup>3</sup> and Posteraro and Gottfried,<sup>4</sup> showed by means of a glycerol lysis test (GLT) that several haemoglobinopathies are associated with a decrease in erythrocyte osmotic fragility, and that this could also be the case with severe chronic renal disease, fetal (cord) red cells, and occasionally with iron deficiency anaemia. Patients with  $\beta$ -thalassaemia trait constituted the largest single group. We have never found that thalassaemia trait in itself results in non-lysis of red cells in the Technicon H6000, and indeed we have tested samples from four patients with  $\beta$ -thalassaemia major none of which failed to lyse normally. It is, however, of note that the only samples reported by Gottfried *et al* to contain haemoglobin C (three patients with haemoglobin SC disease and one with haemoglobin C trait) had the most extreme prolongation of GLT of any of the specimens tested. It would, therefore, appear likely that the Technicon H6000 peroxidase channel fails to lyse only the most osmotically resistance cells, unless there is some other factor related to haemoglobin C with a bearing on erythrocyte membrane stability.

It is noteworthy that the phenomenon occurs with haemoglobin C trait since this would imply that even in the heterozygous state the C haemoglobin exerts a very significant effect on erythrocyte fragility. We have yet to find a single example of erythrocytes containing haemoglobin C which lysed normally in the Technicon H6000. Furthermore, we have found little correlation between red cell morphology and resistance to lysis in the H6000. Several of our cases of haemoglobin C trait have shown minimal or no tendency to target cell formation and would have passed unsuspected but for the striking abnormality in the peroxidase channel. By contrast, we have tested a number of samples with marked degrees of target cell formation from patients with liver disease, none of which has shown any failure of lysis.

The patterns obtained from cases of haemoglobin C trait are sufficiently different from those of haemoglobin SC disease or homozygous haemoglobin C disease to allow their distinction.

In the presence of a normal haemoglobin or mild anaemia, the finding of a positive sickle haemoglobin screen together with failure of lysis of erythrocytes in the H6000 peroxidase channel is highly suggestive of haemoglobin SC disease. Since this diagnosis has potentially important consequences, for instance in the patient about to undergo general anaesthesia, then this rapid diagnostic technique has some clinical value to offset its original observation as an unwanted artefact in the peroxidase channel. The finding *ab initio* of a pattern suggestive of haemoglobin SC or CC disease, which has now occurred on three occasions in our laboratory, is an indication in the first instance to perform a sickle haemoglobin screen (a positive result strongly suggesting the diagnosis of haemoglobin SC disease) and subsequently to proceed to haemoglobin electrophoresis.

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## References

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